Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 21, No. (3) : 2019 : 619-623

ISSN-0972-3005

PREVALENCE OF *CANDIDA* SPECIES IN LEBANESE WATERS

MOHAMMAD HASSAN HALAWI[1]*, HODA YUSEF[1], JAMILA BORJAC[1] AND ZAHER ZEAITER[2]

[1] Biological Sciences Department, Faculty of Science, Beirut Arab University, Debbieh, Lebanon
[2] Life & Earth Sciences Department, Faculty of Sciences, Lebanese University, Beirut, Lebanon

(Received 11 December, 2018; accepted 5 February, 2019)

No detection of *Candida* in Lebanese water supplies have been performed to date. This study could be used to assess the quality of Lebanese water as a possible contaminant of national water supplies, which is currently a major concern for the Lebanese population. This study is a first step of a series of studies that aim at detecting a possible link between the presence of *Candida* in water and the incidence of oral candidiasis, whilst taking into consideration various properties of water to screen for favorable conditions of *Candida* infection spread.

Key words: *Candida, Lebanon, Water, Yeast, Candidiasis, Zinc, Minerals*

Abstract – The prevalence of *Candida* spp. (species) was inspected in 105 water samples (45 potable water, 50 community tap water, and 10 spring water samples), in parallel with the concentration of four different metals (Fe, Cu, Mn, Zn) that are part of *Candida* quorum sensing mechanism. *Candida* spp. were isolated from 84 of 105 (80%) samples. Prevailing species were *Candida Krusei*, isolated from 67 samples, *Candida parapsilosis*, from 38, and *Candida glabrata*, from 34, *Candida tropicalis* from 25, *Candida albicans* from 12, and 5 other species of *Candida*. There was a significant correlation between the concentration of zinc in water and the presence of *Candida* ($P<0.001$). These results are the first to confirm the presence of *Candida* in Lebanese water supplies, and suggest that potable, tap and natural water in Lebanon may be a potential transmission route for *Candida* both in hospitals and community water supplies.

INTRODUCTION

The concept of considering fungi in drinking water as pathogens has been widely ignored, and has not been the focus of researches for long. It is so common for the public to be concerned about water contamination by chemical residues, bacteria, viruses and parasites. Possibly due to the relative acute symptoms of diseases they cause (Hageskal *et al.*, 2009), opposed to the undermined fungal diseases, which could still contribute significantly to human morbidity and mortality (Brown *et al.*, 2012). While bacteria is the most commonly and thoroughly studied pathogenic microorganism in water, fungi have been mostly dismissed, and the quality of water has been accessed in terms of other microbiological parameters, mainly including bacteria, and to a lesser extent, viruses and parasites (Hageskal *et al.*, 2007). Although fungi are the main cause of nosocomial infections, especially among immunocompromised patients, which was the focus of several studies in Lebanon (Basma *et al.*, 2009); (Araj *et al.*, 2015); (El-Zaatari *et al.*, 2002) (Bitar *et al.*, 2014), the lack of sufficient researches focusing on fungal water contamination, may be the reason of the spread of certain diseases that are misdiagnosed or mistreated (Pires Gonçalves *et al.*, 2008).

Recently, the occurrence of fungi in water is being well established (El Hamaoui *et al.*, 2017), the problems related to water contamination by fungi started to gain attention with first the emergence of foul smells detected in water, and then the blockage of pipes and corrosion (Gonçalves *et al.*, 2006). The study of fungi in water has received considerable care in the last decade, due to suspecting its role in nosocomial infections (Hageskal *et al.*, 2009). Lebanese researchers have concentrated lately on the contamination of water by heavy metals (Inhorn *et al.*, 2008), coliforms (Semerjian, 2011) and other enteric bacteria (Harakeh *et al.*, 2006). There have been retrospective studies on the development of *Candida* spp. resistance to antifungal drugs and tracking of prevalence of species in nosocomial isolates (Araj *et al.*, 2015). However, to date, no

***Corresponding author's email:** Mohohalawi@gmail.com

studies that assess the quality of Lebanese water in term of fungal contamination are known. This article presents the first general study of water quality related tothe prevalence of *Candida* spp. contamination among different regions across Lebanon, and a possible link between the concentration of certain metal and the presence of them.

MATERIALS AND METHODS

Sample collections

A total of 105 samples of water, were randomly collected across Lebanon. Samples were selected as: 50 community water samples, 45 potable water samples, and 10 spring water samples. The samples (500 mL) were collected in sterile glass bottles with sodium thiosulfate (10% w/v) and transported to Beirut Arab University - Debbieh laboratories in ice for analysis within 24 hours of collection.

Isolation of Fungi from Culture Media

Sabouraud Dextrose Agar was used for isolation of fungal colonies; it was prepared according to the manufacturer's instructions (Sigma-Aldrich®). The medium was sterilized by autoclaving at 121 °C at a pressure of 15 pound for 15 minutes.

The presence of *Candida* was investigated in 105 water samples, in parallel with physicochemical characteristics of water, that include the pH and the concentration of four key metals (Fe, Cu, Mn, Zn). Forty-five of the samples were potable water, fifty were community tap water, and ten were spring water samples. Water sampled was allowed to flow freely for three minutes through the taps (for tap water), and a solution of sodium hypochloride (100 mg NaOCl/L) was used to sterilize the taps before sampling. Water was then allowed to flow for an extra two minutes. Sterile gloves and long sleeves were worn while collecting to prevent skin flora from contaminating the samples. Water samples were processed using membrane filter technique. Samples (100 mL) were vacuum-filtered, in triplicates, through 47 mm membranes of 0.45 μm pore size (Millipore™). The membranes were then placed in petri dishes (Citotest, Haimen City, China) containing Sabouraud Dextrose Agar supplemented with chloramphenicol (Sigma-Aldrich®). The plates were incubated at 37 °C for 48 hours. The discrete colonies that form on the surface of the membrane were then subcultured to confirmation media. All isolates from the media were sub-cultured into Sabouraud Dextrose Agar (SDA) slants labeled appropriately and refrigerated for further assay.

Identification of *Candida* spp.

Isolated fungi were identified by examining both microscopic, macroscopic, and by chemical characterization. The identification process was carried on preliminary by gram stain and optical microscopy, followed by colony growth patterns and color on SDA. Colonies were finally confirmed by chromogenic agar - Hichrome (Himedia, Bombai, India) according to manufacturer's guidelines.

Measuring the physico-chemical characteristics of water samples

Samples were first analyzed for physical characteristics, appearance, pH, and temperature using Ohaus ST3100-F Starter 3100 Bench Meter (Ohaus Corporation, Parsippany, NJ, USA). Then 5 mL of each sample were filtered on 0.45 μm, 25 -mm diameter, Whatman syringe filter for chemical analysis, which was carried by flame on atomic absorption spectrophotometer (Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometer, Thermo Scientific, Franklin, MA, USA) and double checked on (RayLeigh WFX 210 Series Atomic Absorption Spectrometer, Beijing Rayleigh Instrument Factory, China) for analysis of Mn, Cu, Fe and Zn. The concentration of metals was measured and recorded for each sample independently and the analyses were done in triplicates.

Statistical analysis

Fisher Exact test was used to determine a possible correlation between the type of water sampled (tap or potable) and the presence/absence of *Candida* contamination. Chi-square test was performed on each species separately to test for a link between water sample type and species isolated. The Mann-Whitney U test was used to determine a possible correlation among the concentrations of metals and the presence/absence of *Candida* in samples. The Binary logistic regression coefficient was used to test the correlation between pH-metal levels and possible contamination. Bio-statistical analysis was performed using the statistical SPSS ver. 25 package (2017, SPSS Inc., Chicago, Illinois, USA)

RESULTS AND DISCUSSION

Collected water samples were tested for the presence or absence of *Candida* spp. and the results are summarized in Table 1. A total of 34 isolates of *C. glabrata*, 67 of *C. Krusei*, 38 of *C. parapsilosis*, 25 of *C. tropicalis*, 12 of *C. albicans*, and 5 were recorded as "others" which according to the manufacturer's guidelines could possibly be *Candida glabrata*, *Candida kefyr*, *Candida parapsilosis* or *Candida lusitaniae*, but could not be precisely specified by the followed protocol.

A total of 181 isolates of *Candida* spp. resulted from the tests. Sample 1 through 45 were potable water samples, as samples 46 through 95 were community tap water samples, and sample 96 through 105 were spring water samples.

The prevalence of *Candida* spp. was found to be 80% of the examined water samples. The recovery of *Candida* was significantly higher ($P < 0.05$) in community than in potable water samples. The dominant species were *C. Krusei*, isolated from 63.8%, *C. parapsilosis*, isolated from 36.1%, *C.* glabrata, from 32.3%, *C. tropicalis* from 23.8%, and *C. albicans* from 11.4% of all 105 samples (Table 1).

The concentration of tested metals in water samples is shown in the Figures 1 to 3. There did not appear to be a correlation between the concentration of copper, manganese or iron and the presence of *Candida* spp. However, there was significant negative relation ($P<0.001$) between the concentration of zinc and the presence of *Candida* spp. (Fig. 4).

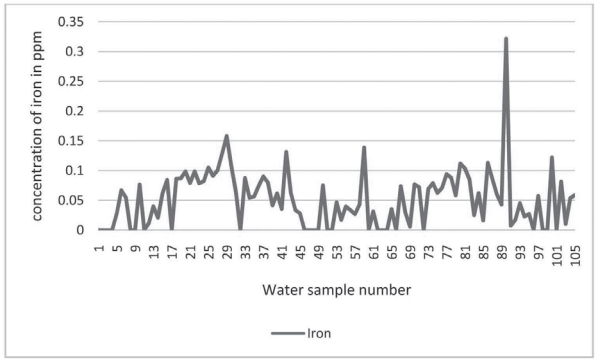

Fig. 1. The concentration of iron (ppm) in water samples.

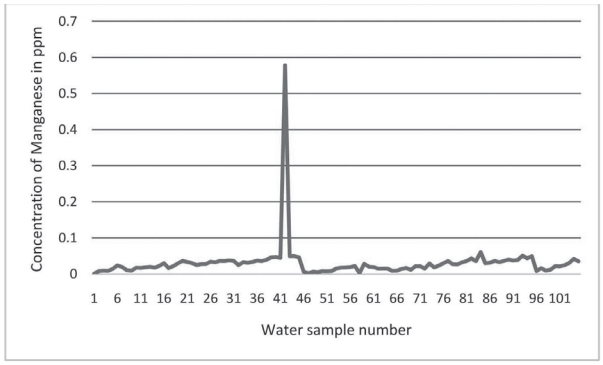

Fig. 2. The concentration of Manganese (ppm) in water samples

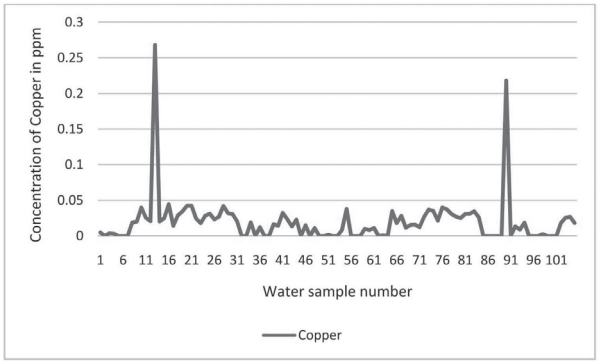

Fig. 3. The concentration of Copper (ppm) in water samples

DISCUSSION

The knowledge concerning the presence of fungal contamination in water and its implications are to date still relatively of narrow limits. The significance and impact on human health is yet to be broadened. Fungal contamination of water is a point of concern when it comes to water physical qualities (taste,

Table 1. Positive (%) water samples for *Candida* species

Candida spp.	Community positive samples (%)	Potable positive samples (%)	Spring positive samples (%)	Total positive samples (%)
C. Krusei	36 (72%)	23 (51.1%)	8 (80%)	67 (63.8%)
C. parapsilosis	23 (46%)	9 (20%)	6 (60%)	38 (36.1%)
C. glabrata	18 (36%)	10 (22.2%)	6 (60%)	34 (32.3%)
C. tropicalis	13 (26%)	9 (20%)	3 (30%)	25 (23.8%)
C. albicans	7 (14%)	3 (6.6%)	2 (20%)	12 (11.4%)

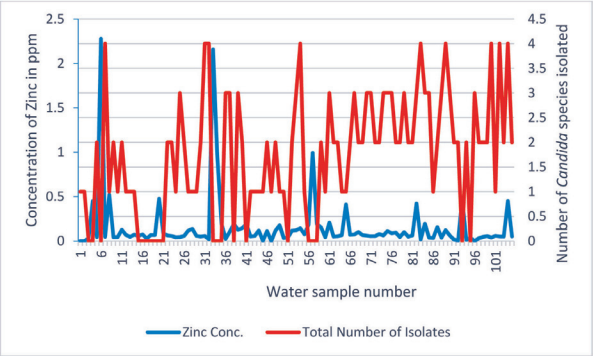

Fig. 4. The concentration of zinc (ppm) and the number of isolates found in each water sample

odor, color), or in areas of high risk (immunocompromised hospitalized patients) (Hageskal *et al.*, 2009).

This study has demonstrated that *Candida* spp. are relatively common in Lebanese distribution and natural water systems. Most importantly may be a route of fungal distribution among the Lebanese population, and immunocompromised patients, which is to be further investigated. Moreover, the current study showed a negative relationship between the concentration of zinc and viability of *Candida* spp. in water samples, as previously proven for *C. albicans*, which could grow in water at concentration of no more than 0.02 ppm (Mowll and Gadd, 1983), and the concentrations of zinc detected in samples vary from 0 to 2.275 ppm. The concentration of mineral metals if compared according to source, will show significant differences, where natural spring water samples appear to have lower concentrations than that of tap and drinking water, mainly due to using metal pipes-plumbing (Sharrett *et al.*, 1982) (Sarin *et al.*, 2004).

The presence of yeast in tap water is not of a less harm than in potable water. Domestic usage of tap water includes – with cultural variations – soaking mouth after teeth brushing, soaking mouth before prayers or after meals or during showers. Moreover, fungal cell or spores are known to be aerosolized into air (Fröhlich-Nowoisky *et al.*, 2009) to affect the respiratory system (Srikanth, 2008). In the case of *Candida*, its presence in tap water may hint a plentiful of diseases which are not limited to oral, respiratory or dermatological (Gonçalves *et al.*, 2006). Further epidemiological studies in Lebanon may link the presence of certain fungi in water to frequent diseases among the Lebanese society.

CONCLUSION

This study highlights the need for future measures concerning water quality all across Lebanon, especially in hospitals and to immunocompromised patients. It is remarkable to include fungal analysis in future water quality testing protocols by Lebanese authorities to levitate the national hygienic status and reduce medical bills, which may need certain regulations and laws. Thereby, local and water suppliers can be informed about the impact of fungal contamination of water.

REFERENCES

Araj, G.F., Rima, G.A. and Aline, Z.A. 2015. *Candida profiles* and antifungal resistance evolution over a decade in Lebanon. *The Journal of Infection in Developing Countries.* 9 : 997-1003.

Basma, R., Ghyda, B., Naim, O. and Khalaf, A.R. 2009. Susceptibility of Candida albicans to common and novel antifungal drugs, and relationship between the mating type locus and resistance, in Lebanese hospital isolates. *Mycoses.* 52 : 141-148.

Bitar, I., Khalaf, R.A., Harastani, H. and Tokajian, S. 2014. Identification, typing, antifungal resistance profile, and biofilm formation of *Candida albicans* isolates from Lebanese hospital patients. *Bio Med Research International:* 2014.

Brown, G., Denning, D., Gow, N., Levitz, S. and Netea, M.A. 2012. Hidden killers: human fungal infections. *Science Translational Medicine.* 4 : 166-165.

El Hamaoui, B., Hamze, M., Hammud, H., Chihib, N., Jama, C., Bakkour, Y., Abbas, Y., El Omar, F. and Monzer, H. 2017. Fast and complete decontamination of bacteria and yeast from water by silica-supported carbon nanoparticles simple filtration. *International Journal of Pharmaceutical Chemistry. 7* : 42-45.

El-Zaatari, M.M., Hulten, K., Fares, Y., Baassiri, A., Balkis, M., Almashhrawi, A. and El-Zaatari, A.F. 2002. Successful treatment of *Candida albicans* osteomyelitis of the spine with fluconazole and surgical debridement: case report. *Journal of Chemotherapy.* 14: 627-630.

Fröhlich-Nowoisky, J., Pickersgill, D. and Després, V.A. 2009. High diversity of fungi in air particulate matter. *Proceedings of the National Academy of Sciences.* 106: 12814-12819.

Gonçalves, A., Paterson, R. and Lima, N. 2006. Survey and significance of filamentous fungi from tap water. *International Journal of Hygiene and Environmental Health.* 209 *:* 257-264.

Hageskal, G., Gaustad, P., Heier, B.and Skaar, I. 2007. Occurrence of moulds in drinking water. *Journal of Applied Microbiology.* 102: 774-780.

Hageskal, G., Lima, N. and Skaar, I. 2009. The Study of Fungi in Drinking Water. *British Mycological Society.*

165-172.

Harakeh, S., Yassine, H. and El-Fadel, M. 2006. Antimicrobial-resistant patterns of *Escherichia coli* and *Salmonella* strains in the aquatic Lebanese environments. *Environmental Pollution.* 143 : 269-277.

Holmes, A., Cannon, R. and Shepherd, M. 1991. Effect of calcium ion uptake on Candida albicans morphology. *FEMS Microbiology Letters*. 187-193.

Ross, I.S. 1982. Effect of copper, cadmium and zinc on germination and mycelial growth in *Candida albicans*. *Transactions of the British Mycological Society.* 78 : 543-545.

Inhorn, M., King, L., Nriagu, J., Kobeissi, L., Hammoud, N., Awwad, J., Abu_Musa, A.A. and Hannoun, A. 2008. Occupational and environmental exposures to heavy metals: Risk factors for male infertility in Lebanon? *Reproductive Toxicology.* 25 : 203-212.

Mowll, J. and Gadd, G. 1983. Zinc Uptake and Toxicity in the Yeasts Sporobolomyces roseus and Sucharomyces cerevisiae. *Journal of General Microbiology.* 1 : 3421-3425.

Pires-Gonçalves, R., Sartori, F., Montanari, L., Zaia, J., Melhem, M., Mendes-Giannini, M. and Martins, C. 2008. Occurrence of fungi in water used at a haemodialysis centre. *Letters in Applied Microbiology.* 46 : 542-547.

Ramanan, N., and Wang, Y. 2000. A High-Affinity Iron Permease Essential for *Candida albicans* Virulence. *Science.* 288 *:* 1062-1064.

Sarin, P., Snoeyink, V., Bebee, J., Jim, K., Beckett, M., Kriven, W. and Clement, J. 2004. Iron release from corroded iron pipes in drinking water distribution systems: effect of dissolved oxygen. *Water Research.* 38 : 1259-1269.

Semerjian, L. 2011. Quality assessment of various bottled waters marketed in Lebanon. *Environmental Monitoring and Assessment.* 172 : 275-285.

Sharrett, A., Carter, A., Orheimt, R. and Feinleib, M. 1982. Daily intake of lead, cadmium, copper, and zinc from drinking water: the Seattle study of trace metal exposure. *Environmental Research.* 28 : 456-475.

Srikanth, P.S. 2008. Bio-aerosols in indoor environment: composition, health effects and analysis. *Indian Journal of Medical Microbiology.* 26 : 302.